A Direct Proton Magnetic Resonance Study of Co^{2+} Complexes with Imidazole, 4-Methylpyridine, Pyridine, Pyrimidine, and Purine in Water–Acetone Mixtures

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Abstract: A proton magnetic resonance study of Co^{2+} complexes with imidazole, 4-methylpyridine, pyridine, pyrimidine, and purine in water–acetone mixtures has been completed. By cooling to temperatures at which ligand exchange is slow, separate pmr signals can be observed for ligand molecules in the Co^{2+} solvation shell and in bulk solvent. Signal integrations and other spectral features indicate that in water–acetone mixtures of the individual bases, the dominant complexes are $Co(Im)_6^{2+}$, $Co(Py)_4(H_2O)_2^{2+}$ and $Co(Py)_3(H_2O)_3^{2+}$, $Co(4\text{-}MePy)_4(H_2O)_2^{2+}$ and $Co(4\text{-}MePy)_3(H_2O)_3^{2+}$, $Co(Pym)_3(H_2O)_3^{2+}$, and $Co(Pur)_3(H_2O)_3^{2+}$. In a water–acetone mixture containing both imidazole and pyrimidine, the only detectable complex is $Co(Im)_6^{2+}$. The presence of the tri- and tetraligand complexes in the pyridine and 4-methylpyridine systems was indicated by multiple spectral patterns at low pyridine concentrations.

Many attempts have been made to gain information concerning the interaction of electrolytes with compounds of biological relevance using proton magnetic resonance (pmr) chemical shift[1–6] and relaxation time[7–12] techniques. The former are based on the fact that the addition of electrolytes to a solution causes a displacement of the ligand molecule pmr signals. Although the interaction site in the molecule can sometimes be deduced, the displacements observed in diamagnetic salt solutions are small, frequently amounting to only a few hertz, and they provide only indirect evidence for the structure of the metal ion–biochemical complex.[1–4] Measurements with paramagnetic ions are much more informative since large chemical shifts (10^4 Hz) and line broadening are produced.[5,6]

Although recent experiments have demonstrated the usefulness of high-frequency (220 MHz) pmr techniques,[13] many studies of high molecular weight biological compounds have been made by the elegant relaxation methods, which observe the behavior of water protons in the presence of Mn^{2+} alone, and Mn^{2+} along with the compound of interest.[7–12] These experiments have led to the determination of interaction sites in several compounds and to a detailed structural picture of the complex. In general, high-resolution methods are more easily applied to compounds of lower molecular weight.

The method used in this study involves the direct observation of the solvation shell of paramagnetic Co^{2+} in the presence of excess ligand. This is made possible by cooling the sample until the ligand exchange rate is less than the frequency difference between signals arising from bulk and complexed molecules. This method has been used to investigate Co^{2+} complexes with methanol,[14] N,N-dimethylformamide,[15] acetonitrile,[16] and other solvents,[17,18] and this study will demonstrate its applicability to Co^{2+}–biochemical systems. The compounds chosen are imidazole, pyrimidine, and purine, species of interest in themselves and as components of more complicated biological molecules, and pyridine and 4-methylpyridine, included for the purpose of comparison. Information obtained includes a quantitative measure of the number of biochemical and water molecules in the Co^{2+} solvation shell, the structure of the complexes, the relative complexing abilities of the ligands, and the ability of two biochemical bases to compete with each other for Co^{2+}, when the bases are present in the same solution.

Experimental Methods

The pyridines, cobalt perchlorate, regular and deuterated (99.5%) acetone, and the biochemicals were reagent grade quality, and they were used as received. The $Co(ClO_4)_2 \cdot 6H_2O$ was analyzed by passage of a portion of a stock solution through a Dowex 50W-X8 cation-exchange column and titration of the resultant acid. In some cases, to remove the water pmr signal, $Co(ClO_4)_2 \cdot 6D_2O$ was prepared by repeated dissolution of the salt in D_2O and evaporation of the excess solvent. The hydrated salt was used in this study to enhance the solubility of the ligands and to introduce the possibility of competition for Co^{2+} by water and the biochemicals. Acetone was used as a diluent to permit studies at low temperatures, to reduce solution viscosity, and to increase the sharpness of the pmr spectrum. Signal overlap was avoided by the use of d_6-acetone when necessary.

The pmr chemical shift and signal area measurements were made on a Varian HA-100 nmr spectrometer, operating on scan mode and using 17-kHz modulation instead of the usual 2.5 kHz, thereby preventing overlap of signals and side bands. Spectra were recorded at probe temperature, and then the samples were cooled in the probe to slow ligand exchange. When separate sets of signals

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could be observed for bulk and complexed ligand, the temperature was varied slightly to maximize the intensity and resolution of the peaks. When not precluded by signal overlap, integrations were made electronically directly with the spectrometer. Analysis of the Co^{2+} solvation shell in solution also was possible by observing spectral changes as a function of ligand concentration.

Experimental Results

In Table I, the number of water and biochemical molecules in the Co^{2+} solvation shell is listed for a range of concentrations in all systems. Probably because of broadening, a signal could not be observed for complexed water molecules in any of the spectra. Since the water signal in all spectra was as sharp as the bulk base proton peaks, it is likely that ligand or proton exchange involving this component was slow enough to permit the observation of a solvation shell peak in the absence of broadening. It is assumed that the water contributions can be calculated by subtracting the value for the organic base from 6.0.

Table I. Co^{2+} Solvation Numbers in Water–Acetone (A) Mixtures of Imidazole (Im), Pyridine (Py), 4-Methylpyridine (4-MePy), Pyrimidine (Pym), and Purine (Pur)

Mole ratios	Temp, °C	Co^{2+} solvation no.[d]
$Co^{2+}:H_2O:Im:A\text{-}d_6$		Im
1.00:6.20:4.00:10.4	−10	4.0
1.00:6.14:5.97:20.2	−30	5.4
1.00:6.20:7.00:19.5[a]	−40	5.4
1.00:6.14:7.28:21.2	−30	6.3
1.00:6.20:7.94:24.4[a]	−40	5.6
1.00:6.23:8.04:20.3	−40	5.7
1.00:6.23:8.37:29.7	−40	5.7
1.00:6.14:9.10:20.0	−30	5.6
$Co^{2+}:H_2O:Py:A$		Py
1.00:6.20:2.96:16.0[b]	−55	2.8
1.00:6.20:3.82:16.0[b]	−50	3.4
1.00:6.20:4.98:15.7[b]	−65	3.7
1.00:6.20:5.91:15.7[b]	−60	3.7
1.00:6.23:7.04:5.84[c]	−40	3.4
1.00:5.69:7.00:16.0	−50	3.2
1.00:6.23:8.10:7.73	−45	3.5
1.00:6.23:9.26:30.6	−50	3.7
1.00:5.69:11.5:12.0	−50	3.4
1.00:9.05:8.08:7.60	−45	3.3
1.00:10.1:8.22:31.9	−50	3.5
1.00:6.23:30.4:0	−50	3.7
$Co^{2+}:H_2O:4\text{-}MePy:A$		4-MePy
1.00:5.69:10.5:15.0	−35	3.6
1.00:9.00:8.30:7.67	−20	3.6
1.00:11.9:8.06:7.72	−20	3.4
1.00:6.23:28.8:0	−40	3.4
$Co^{2+}:D_2O:Im:Pym:A\text{-}d_6$		Pym Im
1.00:6.20:5.87:5.87:19.9	−30	... 5.8
1.00:6.20:9.15:6.83:23.2	−30	... 5.6
$Co^{2+}:D_2O:Pur:A\text{-}d_6$		Pur
1.00:6.20:1.00:21.7	−60	1.0
1.00:9.06:1.99:20.4	−45	2.0
1.00:12.8:2.93:41.8	−65	2.9
1.00:12.2:4.00:30.8	−55	(Broad)
1.00:27.3:4.95:46.3	−45	3.0
1.00:34.8:6.00:47.4	−35	2.5 (broad)
1.00:34.0:6.96:45.7	−25	2.5 (broad)

[a] Samples were prepared with $Co(ClO_4)_2 \cdot 6D_2O$. [b] Samples were prepared with $Co(ClO_4)_2 \cdot 6D_2O$ and acetone-d_6. [c] Samples were prepared with acetone-d_6. [d] It is assumed that the Co^{2+} solvation number is six, and water molecules complete the solvation shell in those systems in which a lower value was measured.

The spectra arising from several of these solutions are shown in Figures 1–7. Although overlap usually precluded an unambiguous area measurement for the entire spectrum, characteristic peaks could always be identified and integrated, resulting in the data of Table I. The integrations were made with a precision of about 10%. In the spectra of the pyridines, the areas of the 3, 4, 5, and methyl pmr signals of complexed ligand were compared to the respective signals of bulk ligand, since these peaks were free of overlap. The total area of the three signals of complexed imidazole was compared to the total bulk imidazole area. The area contributions from water protons or N–H exchange with D_2O also were taken into account. The manner in which the pyrimidine and purine spectra were treated will be described later.

Discussion

The presence of paramagnetic ions usually produces large chemical shift displacements of ligand pmr signals as a result of interactions with the unpaired electrons of the ion. At room temperature, only one set of ligand signals is observed even though the electronic environment of a molecule in a paramagnetic cation solvation shell is markedly different from that of a bulk, noninteracting molecule. The signals arising from these environments are averaged by rapid ligand exchange, which proceeds with a rate constant of about 10^4 sec^{-1} at +25° for most Co^{2+} solutions.[14–16] Exchange can be slowed by cooling the sample until the rate is less than the signal separation corresponding to the bulk and complexed environments, and at this point the separate sets of signals are observable.

The spectra of Figure 1 for a solution of $Co(ClO_4)_2$ and imidazole in a water–acetone-d_6 mixture demonstrate the advantage of this low-temperature pmr method. At +25°, the spectrum is broad and unresolved, in contrast to the spectrum obtained at −40°, where ligand exchange is slow and the bulk and complexed imidazole signals can be observed separately. The assignment of the complexed imidazole peaks was based on (1) an analogy to the pure ligand spectrum of three signals, in which the 4 and 5 protons are equivalent, and (2) the exchange of the N–H proton by dissolution of imidazole in D_2O and the subsequent recovery of the base. When this deuterated imidazole was used, the signal at lowest field in Figure 1 was not present, and it was assigned to the 1 position. It is not clear why the 4 and 5 protons of bound imidazole are still equivalent, but as seen in Figure 1, this appears to be the case. Area measurements and expanded spectra indicate that the three bulk imidazole signals and the bulk water peak are essentially superimposed at high field, reflecting the diminished interaction of these molecules with the paramagnetic ion. Although the resonance positions depend somewhat on solution composition, the relative chemical shifts of Figure 1 are typical of systems containing excess imidazole. The displacements from the corresponding bulk signal are 9000, 6100, and 3450 Hz, respectively, for the 1-, 4- and 5-, and 2-proton signals of complexed imidazole, all to lower field.

As seen in Table I for several imidazole concentrations, signal integrations clearly indicate that there are approximately six molecules of this component in the Co^{2+} solvation shell. Thus, neither acetone nor water

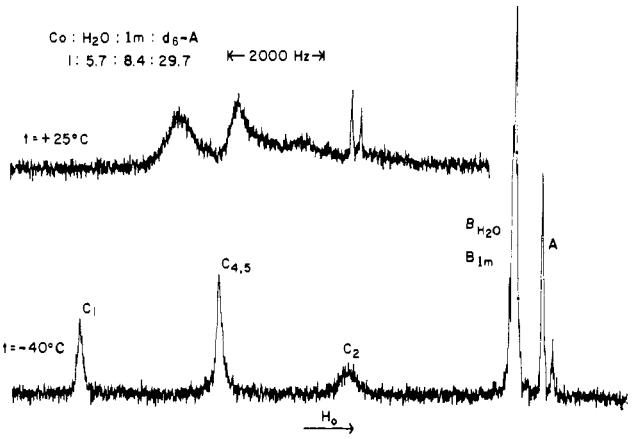

Figure 1. The proton magnetic resonance spectrum of a solution of $Co(ClO_4)_2 \cdot 6H_2O$ and imidazole in acetone-d_6, recorded on a Varian HA-100 nmr spectrometer. The signals arising from bulk water (B_{H_2O}), acetone-d_6 proton impurities (A), bulk imidazole (B_{Im}), and imidazole molecules in the Co^{2+} solvation shell (C) are labeled, with the numerical subscripts identifying the particular imidazole protons. The mole ratio of all species also is given.

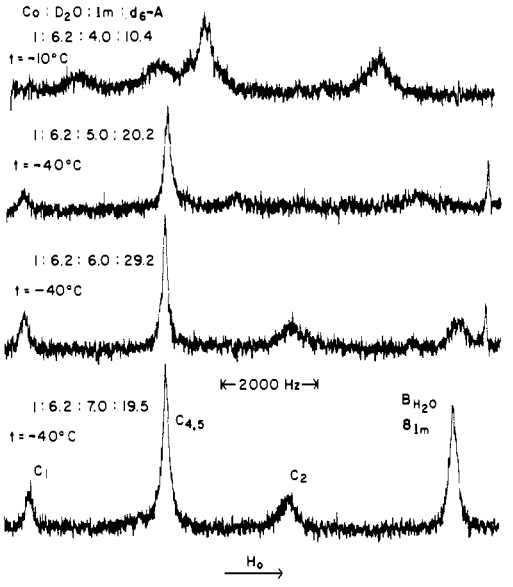

Figure 2. The proton magnetic resonance spectra of four solutions of $Co(ClO_4)_2 \cdot 6D_2O$ in acetone-d_6 containing increasing amounts of imidazole. The signals arising from bulk water (B_{H_2O}), produced by N–H exchange, bulk imidazole (B_{Im}), and imidazole molecules in the Co^{2+} solvation shell (C) are labeled, with the numerical subscripts identifying the particular imidazole protons. Mole ratios also are listed for each solution. The spectra were recorded on a Varian HA-100 nmr spectrometer.

is able to compete with imidazole to any noticeable extent for Co^{2+}, a fact consistent with the relative basic strengths of these species which decrease in the order imidazole > water > acetone.[19] The low solvating ability of acetone also has been well documented in other solvation studies,[20-22] and competition by this component was not expected in any of the systems chosen here.

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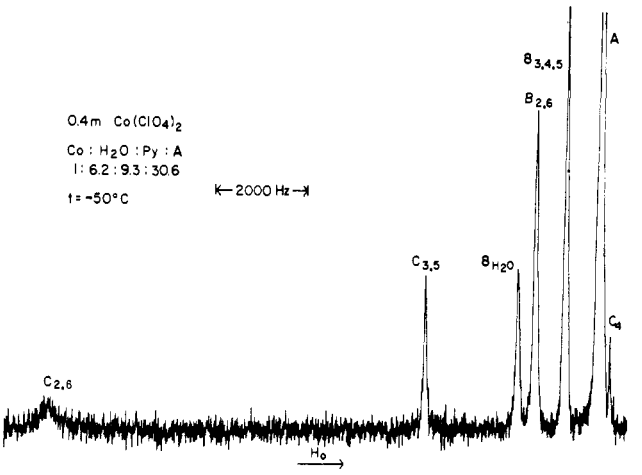

Figure 3. The proton magnetic resonance spectrum of a solution of $Co(ClO_4)_2 \cdot 6H_2O$ and pyridine in acetone, recorded on a Varian HA-100 nmr spectrometer. The signals arising from acetone (A), bulk water (B_{H_2O}), bulk pyridine ($B_{2,6}$, $B_{3,4,5}$), and pyridine molecules in the Co^{2+} solvation shell ($C_{2,6}$, $C_{3,5}$, C_4) are labeled, with the numerical subscripts identifying the particular pyridine protons. The salt molality and the mole ratio of all species also are given.

Although signal integrations are a simple procedure for evaluating the composition of the Co^{2+} solvation shell in imidazole solutions, the spectra of Figure 2 demonstrate a means of accomplishing this for systems producing more complicated spectral patterns, for example, compounds of high molecular weight. The pmr spectra of Figure 2 are those of Co^{2+} solutions in D_2O–acetone-d_6 mixtures containing different amounts of imidazole. At imidazole to Co^{2+} mole ratios of 4, 5, and 6, only one set of signals is observed, and the positions indicate the peaks correspond only to complexed molecules of this base. The broad high-field signal arises from HDO molecules formed by the exchange of N–H protons with D_2O. At an imidazole to Co^{2+} mole ratio of about 7:1, a distinct bulk imidazole signal is evident at its usual high-field position and the spectrum sharpens, presumably because the solvation shell has been exceeded, and the presence of bulk ligand permits a slow exchange to occur. Thus, the presence of $Co(Im)_6^{2+}$ as the dominant species in these solutions is confirmed by signal integrations and the spectral features of Figure 2.

Solutions of $Co(ClO_4)_2$ with pyridine or 4-methylpyridine in water–acetone also exhibit separate pmr patterns for bulk and complexed ligand, as demonstrated by the pyridine spectrum of Figure 3. The chemical shifts of the complexed pyridine signals from those of bulk pyridine are 10,750 and 3100 Hz to lower field for the 2,6 and 3,5 protons, respectively, and 1000 Hz to higher field for the 4 proton. In the spectra of 4-methylpyridine solutions of similar concentrations, the displacements are 10,150 and 2800 Hz for the 2,6 and 3,5 protons of complexed ligand, both to lower field, and 2200 Hz upfield for the methyl peak. With the exception of the methyl signal displacement, the results for both compounds are qualitatively similar to those measured in a previous cobalt halide study.[23] Differences should be expected since the experimental conditions were not the same.

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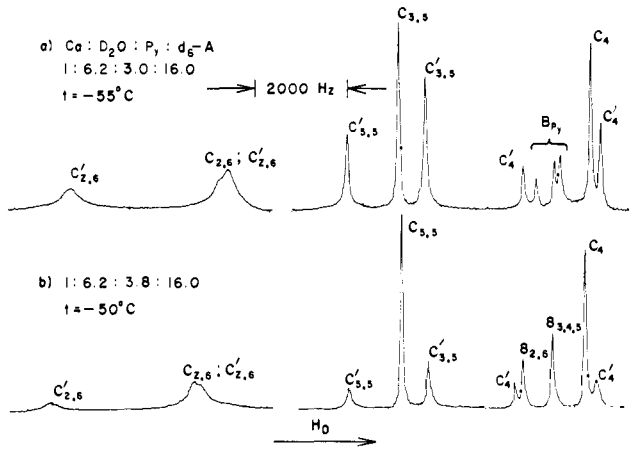

Figure 4. The proton magnetic resonance spectra of solutions of $Co(ClO_4)_2 \cdot 6D_2O$ in acetone-d_6 containing (a) 3 mol and (b) 4 mol of pyridine per mole of Co^{2+}, recorded on a Varian HA-100 nmr spectrometer. The signals arising from bulk pyridine (B_{Py}) and pyridine molecules in the Co^{2+} solvation shell (C and C′) are labeled, with the numerical subscripts identifying the particular protons. The complexes are presumed to be $Co(Py)_3(D_2O)_3^{2+}$ and $Co(Py)_4(D_2O)_2^{2+}$, designated by C′ and C, respectively. The mole ratios of all species also are given.

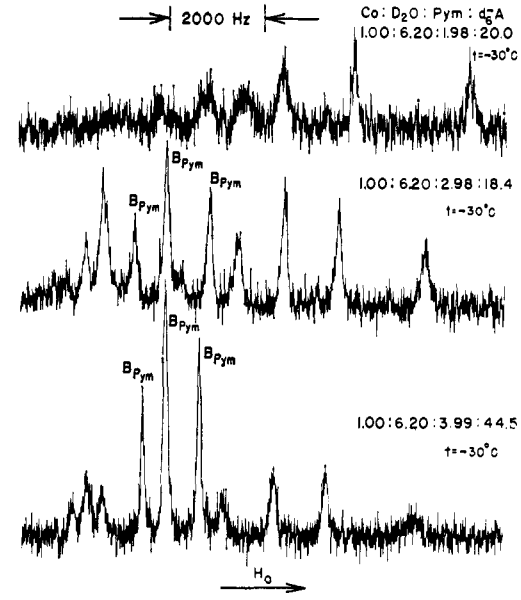

Figure 5. The proton magnetic resonance spectra of solutions of $Co(ClO_4)_2 \cdot 6D_2O$ and pyrimidine in acetone-d_6, recorded on a Varian HA-100 nmr spectrometer. The signals arising from bulk pyrimidine (B_{Pym}) are labeled in the diagram. The mole ratios of all species also are shown.

Spectral integrations for the pyridine and 4-methylpyridine solutions of Table I gave the unexpected result that each Co^{2+} ion is solvated on the average by about 3.5 molecules of these ligands, in contrast to the imidazole complex discussed above. This result is confirmed by the spectra of Figure 4 for solutions of $Co(ClO_4)_3 \cdot 6D_2O$ in acetone-d_6 containing about 3 moles (Figure 4a) and 4 moles (Figure 4b) of pyridine per Co^{2+}. At these low pyridine concentrations, a complicated pattern is observed for the solvation shell signals, unlike the simpler spectrum of Figure 3 for a solution with a large excess of ligand. The spectra of Figure 4 appear to reflect an equilibrium among $Co(Py)_3(D_2O)_3^{2+}$, $Co(Py)_4(D_2O)_2^{2+}$, and a small amount of bulk pyridine. The pattern for each type of complexed pyridine proton involves three signals. A partial superposition in the 2,6-proton region produces only two broad signals, but the skewed nature of one of the signals and its change in shape with pyridine concentration indicate the presence of a third peak. One signal of each three-line pattern would arise from the four pyridine molecules of $Co(Py)_4(D_2O)_2^{2+}$ provided all pyridine molecules lie in the same plane and are equivalent. The remaining two signals, in a 2:1 ratio, would be produced by the dominance of one of the geometrical isomers of $Co(Py)_3(D_2O)_3^{2+}$. If the two isomers were present in large amounts, three signals of equal intensity would be produced, a result which is not observed. Although either isomer is possible, the complex with two pyridine molecules in one plane and the third molecule on an axis perpendicular to this plane would involve less steric hindrance.

Signal integrations also are consistent with this choice of complexes. The area ratio of the signals labeled $C'_{3,5}$, presumably arising from the tripyridine complex, is about 2:1 in Figures 4a and b, and in the spectra of solutions containing 5 or 6:1 mole ratios of pyridine to Co^{2+} (see Table I). The 3,5-proton signals were chosen since they involved the least overlap. Also, the total pyridine contribution to the Co^{2+} solvation shell determined by area measurements can only be interpreted satisfactorily by assuming the simultaneous presence of $Co(Py)_3(D_2O)_3^{2+}$ and $Co(Py)_4(D_2O)_2^{2+}$. For the systems containing 3, 4, 5, and 6 mol of pyridine per Co^{2+}, the calculated average numbers of complexed pyridine molecules are 2.6, 3.6, 3.8, and 3.8, respectively, which agree within 10% to the measured values listed in Table I. Other combinations of complexes gave poor agreement between the calculated and measured values. It is significant that even with only 3 mol of pyridine per Co^{2+} present, the choice of these two complexes predicts the presence and the amount of bulk pyridine, identified in Figure 4a.

As anticipated, an increase in the pyridine concentration results in an increase of $Co(Py)_4(D_2O)_2^{2+}$ and a decrease of $Co(Py)_3(D_2O)_3^{2+}$ (see Figure 4a and b). However, the average number of pyridine molecules complexed, about 3.5, over the range of solvent and ligand composition shown in Table I attests to the stability of both species and to the instability of higher pyridine complexes. Since pyridine should easily displace the less basic water molecules from the Co^{2+} shell, higher complexes must involve severe steric hindrance and electronic repulsion. The 4-methylpyridine data of Table I indicate that the same situation probably prevails in Co^{2+} complexes of this compound. Conversely, the smaller size and the decreased aromatic character of imidazole minimize these problems and permit the formation of $Co(Im)_6^{2+}$.

Although the complexity of the pmr spectra of Figure 5 for Co^{2+}–pyrimidine complexes in D_2O–acetone-d_6 mixtures probably reflects a phenomenon similar to that which occurs in the pyridine solutions, this aspect was not pursued in any detail. Rather an attempt was made to deduce only the average number of pyrimidine molecules complexed per Co^{2+} in solutions containing excess ligand. In Figure 5, a set of broad signals is observed for the 2:1 pyrimidine:Co^{2+} solution, the broadness

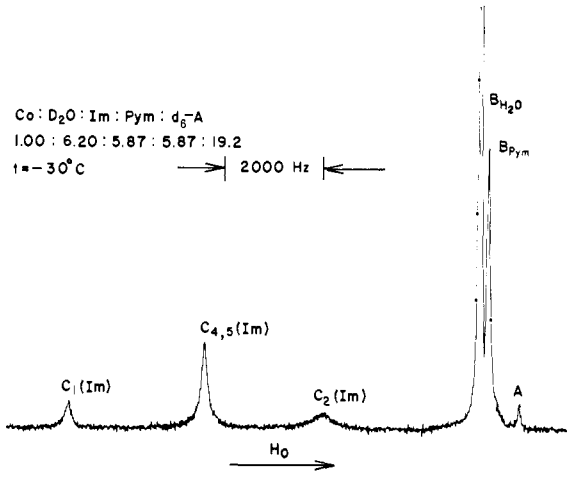

Figure 6. The proton magnetic resonance spectrum of a solution of $Co(ClO_4)_2 \cdot 6D_2O$, imidazole, and pyrimidine in acetone-d_6, recorded on a Varian HA-100 nmr spectrometer. The signals arising from bulk water (B_{H_2O}), produced by N–H exchange, bulk pyrimidine (B_{Pym}), and imidazole molecules in the Co^{2+} solvation shell (C) are labeled in the diagram, with numerical subscripts indicating the particular imidazole protons. The mole ratio of all species also is given.

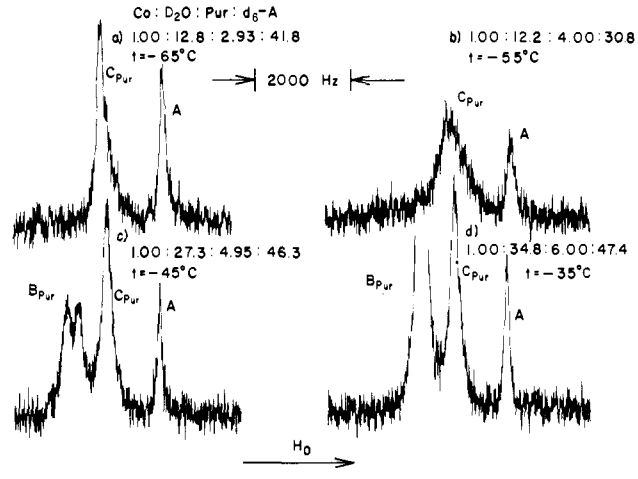

Figure 7. The proton magnetic resonance spectra of solutions of $Co(ClO_4)_2 \cdot 6D_2O$ and purine in acetone-d_6, recorded on a Varian HA-100 nmr spectrometer. The signals arising from acetone proton impurities (A), bulk purine (B_{Pur}), and purine molecules in the Co^{2+} solvation shell (C_{Pur}) are labeled in the diagram. The mole ratios of all species also are shown.

indicating that essentially all base is complexed. As the mole ratio is increased to 3:1, bulk pyrimidine signals appear in a roughly 1:2:1 area ratio, and they increase noticeably in intensity in the 4:1 pyrimidine:Co^{2+} system. Since the bulk ligand peaks of Figure 5 are much larger than in the pyridine spectra of similar concentrations (Figure 4a and b), the Co^{2+}–pyrimidine complex must contain fewer than 3.5 pyrimidine molecules on the average. A value of 2 to 3 would seem reasonable in view of the spectra of Figure 5. Although pyridine and pyrimidine are about equal in size and aromatic character, this difference in complexing tendency may be due to the lower relative basicity of pyrimidine[19] and the increased repulsion produced by the unshared electron pair on the second nitrogen of this molecule.

The results of a direct competition between imidazole and pyrimidine for Co^{2+} are presented in Table I and Figure 6. In this figure, the spectral pattern of complexed imidazole molecules (see Figures 1 and 2) is clearly evident for the solution containing this component and pyrimidine each in a 6:1 mole ratio to Co^{2+}, but signals of complexed pyrimidine are not noticeable. The results of area integrations are shown in Table I and they also are consistent with the conclusion that $Co(Im)_6^{2+}$ is the dominant species in these systems. This fact is not surprising in view of the results obtained with each of these species individually. However, this experiment illustrates that an unambiguous estimation of complexing abilities can be obtained by the direct study of the complex pmr signals. This is a more quantitative approach than the measure of chemical shift displacements of a few hertz for the averaged, room-temperature spectrum.

Finally, a study of Co^{2+}–purine complexes was made since this compound is of wide biological importance and since its structure comprises that of both imidazole and pyrimidine. As seen in Figure 7, the spectra of these systems are not well resolved, but area integrations and the concentration dependence of the signals provided an estimate of the composition of the complex. From the spectra of Figure 7 and those of other systems containing higher ligand concentrations, the signals of uncomplexed purine were readily identified. These bulk signals are evident only at mole ratios of purine:Co^{2+} of 5:1 or greater, although they may be obscured by overlap in the 4:1 system. In fact, a likely cause of the excessive broadening in the spectrum of this 4:1 purine:Co^{2+} solution may be the presence of bulk ligand and subsequent exchange. Signal overlap also complicated the area measurements, which indicated that about three molecules of purine are bound per Co^{2+}. These results are analogous to those obtained from the pyridine study, and it is reasonable to assume that the complexes are similar in both cases. Thus, an average of three to four molecules of purine are probably bound per Co^{2+}, although signal resolution precluded an unambiguous structure determination of the complex. The large size of the purine molecule and the electronic repulsion arising from its aromatic character offer the best explanation for the inability of this compound to saturate the Co^{2+} solvation shell to a maximum of six molecules.

These results demonstrate that direct pmr measurements by the method described here provide a quantitative estimate of the extent of interaction between metal ions and compounds of biological importance. Although the technique is limited to systems of reasonable signal resolution, a wide range of such compounds should lie within the scope of study.

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